FORESTRY

FRDA REPORT 058

FACTORS AFFECTING CONE AND SEED PRODUCTION IN CONTAINERIZED INTERIOR SPRUCE

by
ANNA M. COLANGELI*
MARGARET D. DAWKINS*
JOHN N. OWENS*

FOREST RESOURCE DEVELOPMENT AGREEMENT

Canada

BC

FRDA REPORT 058

FACTORS AFFECTING CONE AND SEED PRODUCTION IN CONTAINERIZED INTERIOR SPRUCE

by

ANNA M. COLANGELI*
MARGARET D. DAWKINS*
JOHN N. OWENS*

* Department of Biology
University of Victoria
Victoria, B.C.
V8W 2Y2

Departmental Representative:

Jack Sutherland
Forestry Canada
Pacific Forestry Centre
506 W. Burnside Road
Victoria, B.C.
V8Z 1M5

February 1989

This report has been reviewed by Forestry Canada and approved for distribution. Approval does not necessarily signify that the contents reflect the views and policies of Forestry Canada. Mention of trade names or commercial products does not constitute recommendation or endorsement for use.

ISSN 0835-0752

Canadian Cataloguing in Publication Data

Colangeli, Anna Maria, 1956-
Factors affecting cone and seed production in containerized interior spruce

(FRDA report, ISSN 0835-0752; 058)

On cover: Canada/B.C. Forest Resource Development Agreement
Bibliography: p.
ISBN 0--662-16756-2
Fo29-19/058E

1. Engelmann spruce - British Columbia - Seeds 2. White spruce - British Columbia - Seeds. 3. Engelmann spruce - British Columbia - Reproduction. 4. White spruce - British Columbia - Reproduction. 5. Engelmann spruce - British Columbia - Pollen. 6. White spruce - British Columbia - Pollen. I. Dawkins, Margaret D. II. Owens, John N., 1936- . III. Canada. Forestry Canada. I Forest Resource Development Agreement (Canada). V. Title. VI. Series.

SD397.S77C64 1989 634.9'752562 C89-092047-8

ABSTRACT

Forcing pollen-cone buds at elevated temperatures (15-17°C/10°C, day/night temperatures) starting at reproductive bud swell in early March increased the rate of pollen-cone bud and pollen development relative to non-forced (Control) buds. Forced ramets shed pollen 1 to 2 weeks earlier than non-forced ramets. Approximately twice as many filled seed were produced after pollination with non-forced pollen than forced pollen suggesting that forcing reduced pollen quality. The reduction in pollen quality was not reflected in carbohydrate levels within the mature pollen. Recommendations suggested by the study would be not to start pollen forcing at early developmental stages.

Conelet abortion at pollination, in environmental chambers occurred when conelets were subjected to -6°C for 1 or 3 nights. Conelet abortion was not observed when ramets were maintained at 2 or -2°C for 1 or 3 nights, suggesting that as with field-grown interior spruce, conelet abortion at pollination is not a prevalent problem.

Low seed efficiencies were observed in both studies, that could not be explained by the different treatments. The potential causes of low seed yield in containerized interior spruce ramets is discussed.

INTRODUCTION

In the past few years there has been a growing interest in containerized orchards for acceleration of both breeding and the production of genetically improved seed (Ross *et al.* 1986). While the promotion of reproductive buds on containerized propagules of interior spruce (*Picea engelmannii* and *P. glauca*) has been very successful it was also found that elevated polyhouse temperatures affected pollen cone development (Ross 1988), seed cone size (Ross 1985; Ross 1988) and filled seed per cone (Ross 1988).

Pollen forcing (pollen development at above ambient temperatures) to obtain pollen early for controlled crosses or supplemental pollinations is necessary, yet very little is known about the effect of pollen forcing on pollen development, germinability, vigor or seed set. Conelet abortion at pollination due to low temperatures, while not a common occurrence in field-grown interior spruce clones, is prevalent in other species. The effect of low temperatures on seed efficiency in containerized interior spruce is not known.

In order to help identify the possible causes of low seed set in containerized interior spruce two studies were conducted aimed at two stages in the reproductive cycle. The first study is designed to observe and examine the effects of forcing on pollen development and fertilizing potential (prefertilization factors). The second study is to determine the effect of three different temperatures at pollination on cone abortion and final seed efficiency.

METHODS

Study 1: Ramets from four clones were divided into control and treatment groups. The treatment trees were placed in a heated greenhouse with day temperatures of 15-17°C and night temperatures of 10°C on March 5, 1986, at or before meiosis for clones 3, 21 and 52 and just after meiosis for clone 1675, as determined by squash preparations. The control group was placed in an open greenhouse at ambient temperatures and natural photoperiod.

Pollen cone collections started March 6 and continued biweekly until all cones had shed pollen. On each collection date the cones were categorized into the following phenological stages prior to dissection:

(1) quiescent pollen cone
(2) pollen-cone bud swelling
(3) pollen-cone bud bursting
(4) pollen cone 25-50% emerged from bud scales
(5) pollen cone 50-75% emerged from bud scales
(6) pollen cone fully emerged from bud scales
(7) axis elongation
(8) pollen shed

Each cone was dissected where part of the cone was fixed for microscopic examination and the rest frozen in liquid nitrogen for carbohydrate (glucose, sucrose, starch) analysis.

Mature pollen collected from the clones was used to determine the pollen fertilizing potential. Receptive conelets from three clones were pollinated at the Skimikin Seed Orchard, Salmon Arm, B.C. in May 1986. Four to eight cones per clone were isolated using windowed pollination bags. The conelets were brush pollinated on either 15 or 16 May and again on 18 May with the pollen collected from the four control and treated clones (8 pollinations). The pollination bags were left on the trees until cone closure and then replaced with mesh insect bags. The mature cones were collected prior to seed shed in the fall for determination of total number of fertile scales and number of filled seed. Seed potential (SP) was determined by multiplying the number of fertile scales by two because each fertile scale bears two ovules and each can potentially develop into a filled seed. Seed efficiency (SEF) was calculated by dividing the number of filled seed by Sp and multipling by 100%.

Study 2: During the spring of 1987, conelets on 6 ramets from each of three clones were brush-pollinated, twice. The first pollination was conducted after the cones had emerged through the bud scales and the ovuliferous scales had reflexed and the second pollination conducted 7 days later. One ramet of each of the three clones was placed into growth chambers maintained at 10°C day temperatures and at 2°C, -2°C or -6°C night temperatures for either one or three days. Following cold treatment the ramets were returned to ambient conditions in a frost protected area. Conelet abortion was assessed two weeks after the final treatment. The surviving cones were harvested in the fall for seed efficiency determinations as previously described.

RESULTS

Study 1: Forcing increased the rate of pollen-cone bud development and the rate of pollen development within the cone bud. Forced trees shed pollen one week earlier than the control trees in clone 1675 and 2 weeks earlier in clones 3 and 52. This difference may be related to the fact that clone 1675 had completed meiosis when treatments began, whereas clones 3 and 52 were still at the microspore mother cell stage.

Results of the carbohydrate analysis were inconsistent. Glucose and sucrose levels of control buds were significantly higher than forced buds at stages 6 and 7 in clone 52. Control buds had significantly higher glucose levels at the 4-celled stage of clone 3, and significantly higher sucrose levels at the 4- and 5-celled stage of clone 52. No significant differences between forced and non-forced cone buds were found at any stage in clone 1675.

SEFs were significantly different for three of the four clones (3, 52 and 1675) pollinated with forced and control (non-forced) pollen where lower SEFs were produced by forced pollen (Table 1). No differences in SEF were found in clone 21.

Study 2: Conelet abortion was almost zero in the spruce ramets subjected to 2°C or -2°C temperatures for 1 and 3 nights. All ramets maintained at -6°C for one or three nights suffered considerable conelet abortion (Table 2). Clone 28 was slightly more resistant to the cold treatments than clones 3 and 21.

Cone abortion rates between pollination and cone maturity were high in all six ramets of clone 3 regardless of treatment (Table 3). This was not observed in clones 21 or 28. The factors resulting in the high abortion rate in clone 3 have not yet been determined.

SP was not affected by treatment at pollination (Table 4). This is not surprising because the ovuliferous scales and ovules are initiated before dormancy and the total number determined before pollination. However, filled seed and seed efficiency was affected by treatment for clone 3, but not clones 21 and 28 (Table 4).

DISCUSSION

With the exception of clone 21, forcing the pollen during development reduced the pollens' fertilizing potential as determined by seed efficiency. This was not reflected in the carbohydrate levels within the pollen. Many other factors could have reduced the pollens' fertilizing potential. Mature pollen grains contain enzymes and isozymes encompassing all six classes of enzymes (Stanley and Linskens 1974), plant growth regulators (Sweet and Lewis 1969, 1971; Kamienska and Pharis 1975; Kamienska *et al.* 1976a, 1976b), ATP (Ching *et al.* 1975), soluable proteins (Pettitt 1985) and lipids (Andrikopoulous *et al.* 1985), all of which may play an important role in pollen germination and pollen tube development. Any of these substances could be affected by the elevated temperatures and accelerated rate of development. Also, the nucellar tissue through which the pollen tube grows, is ladden with starch which may be utilized by the pollen tubes. Therefore, pollen may only require enough starch to initiate tube growth. Subsequent growth may result from starch reserves in the nucellus.

Heat treatments were started at the onset of reproductive bud swelling, in the spring coinciding with meiosis in 3 of the 4 clones. In pollen, meiosis is known to be sensitive to temperatures not considered to be excessive for vegetative growth (Chira 1965; Eriksson *et al.* 1970; Jonnsson 1974; Luomajoki 1977). Elevated temperatures may be less detrimental later in development when cell divisions are not occurring. Interior spruce undergoes several weeks of pollen development when cell walls develop and reserves accumulate (Owens and Molder 1977; Singh and Owens 1980). High temperatures may not adversely affect these. The next phase of cell division does not occur until later in the spring when high ambient temperatures are common. A recommendation from this study is not to force pollen at early developmental stages (eg. meiosis). Further studies are required to determine if forcing at later stages of development also affects pollen quality.

Conelet abortion at pollination is not a prevalent problem in field-grown interior spruce. The same was found in this study for containerized interior spruce. The conelets subjected to 2° and -2°C temperatures for 1 or 3 nights experienced no cone abortion within the next 2 weeks, while conelets subjected to -6°C temperatures for 1 or 3 nights suffered high conelet abortion within 2 weeks of treatment. Clonal variation was observed, where clone 28 was less frost susceptible than clones 3 or 21. Clone 3 had almost complete cone abortion during late cone development regardless of the treatment at pollination. The exact causes of cone abortion in this clone could not be determined.

Very few filled seed were produced in all clones and treatments, even though the cones were biologically capable of yielding 140 or more filled seed per cone. There was a general decreasing trend in SEF in cones treated at 2°, -2° and -6°C, therefore temperature at pollination may have more subtle effects but may not account for all the empty seeds found. The SEFs produced in the first study were also low, averageing 24 and 11%, respectively for the nonforced (control) and forced pollen. The low SEF observed after controlled crosses in interior spruce may be due to the pollination mechanism. Earlier studies in spruce showed that all ovules are not receptive at once and pollination drops are exuded acropetally in the cone over several days (Owens and Blake 1985; Owens *et al.* 1987). Therefore, two pollinations several days apart may not pollinate all ovules. Recent studies in *Larix* (Kozinski 1987), *Tsuga* (Colangeli 1989), and *Thuja* (Colangeli and Owens 1989; Owens *et al.* 1989) have demonstrated that there are many stages during the reproductive cycle of a conifer that result in empty seed. These include inadequate pollination, low pollen vigor and viability and embryo and megagametophyte abortion. Any or all of these factors may have reduced the final seed yield in interior spruce. Further research is required to determine what factors are most important in reducing potential seed yield in interior spruce.

CONCLUSIONS AND RECOMMENDATIONS

In summary, interior spruce pollen forced at above ambient temperatures early in development reduces seed set; multiple pollinations with pollen of known high quality may increase seed efficiency; and, temperatures at or just below freezing at pollination do not cause cone abortion in containerized interior spruce. The high cone abortion rate observed in one clone, regardless of treatment, may in the future, warrant the screening of clones before they are used in seed production and breeding programs.

ACKNOWLEDGEMENTS

This study was funded by Forestry Canada under the Canada-British Columbia Forest Resource Development Agreement (1985-1990). This report has been reviewed by Forestry Canada and approved for distribution. This approval does not necessarily signify that the contents reflect the views or policies of Forestry Canada.

REFERENCES

ANDRIKOPOULOS, N.K., SIAFAKA-KAPADAI, A. DEMOPOULOS, C.A., and KAPOULAS, V.M. 1985. Lipids of *Pinus halipensis* pollen. Phytochem. 24: 2953-2957.

CHING, T.M., RANZONI, M.W. and Ching, K.K. 1975. ATP content and pollen germinability of some conifers. Plant Sci. Lett. 4: 331-333.

CHIRA, E. 1965. On some biological questions concerning *Picea escelsa* (Lam) Link. pollen. Biologia 20: 641-653.

COLANGELI, A.M. 1989. Sexual reproduction in western hemlock. Ph.D. Dissertation, University of Victoria, Victoria, B.C. 169 p.

COLANGELI, A.M. AND OWENS, J.N., 1989. Seed production in western redcedar (*Thuja plicata* Donn): I. The role of pollination mechanism and time of pollination on pollination efficiency. Can. J. For. Res. (submitted).

ERIKSSON, G., EKBERG, I. and JOHSSON, A. 1970. Meiotic investigations in pollen mother cells of Norway spruce cultivated in a plastic green hous. Hereditas 66: 1-20.

JONNSSON, A. 1974. A study on the temperature response of pollen mother cells in Norway Spruce. Stud. For. Suec. 116: 1-32.

KAMIENSKA, A., DURLEY, R.C. and PHARIS, R.P. 1976a. Isolation of gibberellins A_3, A_4 and A_7 from *Pinus attenuata* pollen. Phytochem. 15: 421-424.

____ 1976b. Endogenous gibberellins of pine pollen. III. Conversion of 1,2-[^{3}H]GA_4 to Gibberellins A_1 and A_{34} in germinating pollen of *Pinus attenuata* Lemm. Plant Physiol. 58: 68-70.

KAMIENSKA, A., and PHARIS, R.P. 1975. Endogenous gibberellins of pine pollen II. Changes during germination of *Pinus attenuata*, *P. coulteri*, and *P. ponderosa* pollen. Plant Physiol. 56: 655-659.

KOZINSKI, G. 1987. Megagametogenesis, fertilization, and embryo development in *Larix decidua*. Can. J. For. Res. 16: 1301-1309.

LUOMAJOKI, A. 1977. Effects of temperature on spermatophyte male meiosis. Hereditas 85: 33-48.

OWENS, J.N. and BLAKE, M.D. 1985. Forest tree seed production. A review of literature and recommendations for future research. Can. For. Serv. Inf. Rep. P1-X-53.

OWENS, J.N., COLANGELI, A.M., and MORRIS, S.J. 1989. Seed production in western redcedar (*Thuja plicata* Donn): II. The effect of self, cross and no pollination on ovule, embryo, seed and cone development. Can. J. For. Res. (submitted).

OWENS, J.N., and MOLDER, M. Bud development in *Picea glauca*. II. Cone differentiation and early development. Can. J. Bot. 55: 2746-2760.

OWENS, J.N., SIMPSON, S.J., and CARON, G. 1987. The pollination mechanism in Engelmann spruce (*Picea engelmannii* Parry). Can. J. For. Res. 10: 564-572.

PETTITT, J.M. 1985. Pollen tube development and characteristics of the protein emission in conifers. Ann. Bot. 56: 379-397.

ROSS, S.D. 1985. Promotion of flowering in potted *Picea engelmannii* (Perry) grafts: Effects of heat, drought, gibberellin $A_{4/7}$ and their timing. Can. J. For. Res. 15: 618-624.

____ 1988. Pre- and post-pollination polyhouse environment effects on pollen and seed development in potted *Picea engelmannii* grafts. Can. J. For. Res. 18: 623-627.

ROSS, S.D., EASTHAM, A.M., and BOWER, R.C., 1986. Potential for container seed orchards. *In* Proceedings -- Conifer Tree Seed in the Inland Mountain West. *Edited by* R.C. Shearer. USDA For. Serv. Gen. Tech. Rep. INT-203. pp. 180-186.

SINGH, H. and J.N. OWENS. 1981 Sexual reproduction of Engelmann spruce (*Picea engelmannii*). Can. J. Bot. 59: 793-810.

STANLEY, R.G., and LINSKENS, H.F. 1974. Pollen: Biology Biochemistry Management. Springer-Verlag New York. 307 pp.

SWEET, G.B., and LEWIS, P.N. 1969. A diffusible auxin from *Pinus radiata* pollen and its possible role in stimulating ovule development. Planta 89: 380-384.

____ 1971. Plant growth substances in pollen of *Pinus radiata* at different levels of germination. N.Z. J. Bot. 9: 146-156.

Table 1: Seed efficiencies produced by non-forced (control) and forced pollen collected from four interior spruce clones.

CLONE	SEED EFFICIENCY (±SE) NON-FORCED	FORCED
3	35.7 (4.0)	15.8 (2.8)*
21	31.7 (7.0)	25.3 (4.1)
52	16.8 (2.1)	0.7 (0.4)*
1675	12.6 (1.7)	3.9 (0.7)*
MEAN	24.2	11.4*

* Significant difference between seed efficiencies produced by non-forced and forced pollen ($p<0.05$).

Table 2: The effect of one and three nights of near freezing and freezing temperatures on three interior spruce clones.

Temp		Clone/Ramet	Aborted Cones	Non-aborted Cones	Total	Percent Abortion[1]
10°/ 2°C	(1)[2]	3-25	1	29	30	3
	(3)	3-18	0	23	23	0
	(1)	21-27	0	17	17	0
	(3)	21-15	0	24	24	0
	(1)	28-05	0	31	31	0
	(3)	28-07	0	31	31	0
10°/-2°C	(1)	3-40	0	14	14	0
	(3)	3-34	0	27	27	0
	(1)	21-49	0	21	21	0
	(3)	21-03	0	25	25	0
	(1)	28-13	0	24	24	0
	(3)	28-03	0	41	41	0
10°/-6°C	(1)	3-13	21	2	23	91
	(3)	3-27	26	6	32	81
	(1)	21-11	20	2	22	91
	(3)	21-26	25	5	30	83
	(1)	28-22	15	7	22	68
	(3)	28-30	20	9	29	69

[1]At pollination
[2]Number of nights in the growth chambers.

Table 3: Cone abortion between pollination and cone maturity.

Temp		Clone/Ramet	Aborted Cones	Non-aborted Cones	Total	Percent Abortion
10°/ 2°C	(1)[1]	3-25	25	4	29	86
	(3)	3-18	21	2	23	84
	(1)	21-27	0	17	17	0
	(3)	21-15	0	24	24	0
	(1)	28-05	0	31	31	0
	(3)	28-07	0	31	31	0
10°/-2°C	(1)	3-40	12	2	14	86
	(3)	3-34	21	6		78
	(1)	21-49	0	21	21	0
	(3)	21-03	0	25	25	0
	(1)	28-13	1	23	24	4
	(3)	28-03	0	41	41	0
10°/-6°C	(1)	3-13	2	0	2	100
	(3)	3-27	6	0	6	100
	(1)	21-11	1	1	2	50
	(3)	21-26	5	0	5	0
	(1)	28-22	7	0	7	0
	(3)	28-30	9	0	9	0

[1]Number of nights in the growth chambers.

Table 4: Mean (±SE) seed potential (SP), filled seed (FS) and seed efficiencies (SEF) for non-aborted seed cones (N).

Temp		Clone/Ramet	N	SP	FS	SEF
10°/ 2°C	(1)[1]	3-25	4	124 ± 5	16.3 ± 4.3	13.4 ± 3.5
	(3)	3-18	2	131 ± 4	16.5 ± 2.5	12.7 ± 2.7
	(1)	21-27	10	153 ± 5	15.2 ± 1.6	10.2 ± 1.2
	(3)	21-15	10	160 ± 4	24.2 ± 3.1	15.1 ± 2.3
	(1)	28-05	10	191 ± 4	19.8 ± 2.3	10.3 ± 1.2
	(3)	28-07	10	194 ± 8	24.0 ± 2.6	12.4 ± 1.3
10°/-2°C	(1)	3-40	2	140 ± 8	3.5 ± 0.4	2.2 ± 0.1
	(3)	3-34	6	127 ± 6	7.8 ± 2.9	6.1 ± 2.9
	(1)	21-49	10	150 ± 3	25.7 ± 3.2	17.1 ± 2.2
	(3)	21-03	10	169 ± 4	19.8 ± 2.3	11.7 ± 1.2
	(1)	28-13	10	212 ± 5	20.4 ± 2.2	9.7 ± 1.0
	(3)	28-03	10	183 ± 9	16.3 ± 1.6	8.9 ± 1.2
10°/-6°C	(1)	3-13	0	--	--	--
	(3)	3-27	0	--	--	--
	(1)	21-11	1	160	13	8.1
	(3)	21-26	5	146 ± 5	12.4 ± 4.1	8.9 ± 3.1
	(1)	28-22	7	176 ± 9	21.1 ± 1.6	12.1 ± 1.0
	(3)	28-30	9	190 ± 5	15.7 ± 2.4	8.4 ± 1.2

[1]Number of nights in the growth chambers.